Selenium intake, age, gender, and smoking in relation to indices of selenium status of adults residing in a seleniferous area[1-3]

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ABSTRACT Duplicate meals, serum, whole blood, and toenails were collected every 3 mo for 1 y from a group of 44 free-living adults residing in high-selenium areas of South Dakota and Wyoming to assess the relation of selenium intake to indices of selenium status. The average selenium values for the group were as follows: dietary intake, 174 ± 91 μg/d ($\bar{x}$ ± SD), 2.33 ± 1.08 μg/kg body wt; serum, 2.10 ± 0.38 μmol/L; whole blood, 3.22 ± 0.79 μmol/L; and toenails, 15.2 ± 3.0 nmol/g. Selenium intake (μg/kg body wt) was strongly correlated (all values, $P < 0.01$) with selenium concentration of serum ($r = 0.63$), whole blood ($r = 0.62$), and toenails ($r = 0.59$). Men and women had similar mean values of serum, whole blood, and toenail selenium despite higher selenium intakes in men. Smokers had lower tissue selenium concentrations than did nonsmokers due, at least in part, to lower selenium intake. Age was not associated with tissue selenium content. Of the variables examined selenium intake was clearly the strongest predictor of tissue selenium concentration. *Am J Clin Nutr* 1990;52:858–62.

KEY WORDS Selenium intake, selenium status, humans

Introduction

Low serum selenium has been associated with increased risk of cardiovascular disease and cancer in some epidemiological investigations (1–3). Low serum selenium was linked, by inference, to low selenium intake, thus stimulating interest in initiating selenium supplementation studies (4). Burk (5), however, cautioned that differences in serum selenium might be attributed to other dietary factors, including the chemical form of selenium in the diet. Other investigators (6–8) reported that age, gender, race, alcohol, smoking, and concurrent disease also influenced serum selenium concentration.

Selenium supplementation studies clearly demonstrate a direct relation between selenium intake and blood selenium content (9, 10). International data also indicate a strong positive correlation between selenium intake and blood selenium concentration (11). Within a specific country or geographic region, blood selenium is assumed to reflect dietary intake primarily, and other factors are considered to be of secondary importance. A few investigators (12, 13) examined the relation between calculated selenium intake and blood selenium content of individuals consuming self-selected diets. In those studies selenium intake was not found to be predictive of blood selenium concentration.

Because the selenium content of foods varies widely, selenium intake cannot be estimated readily from standard food tables (14). A more precise determination of selenium intake can be made if diet composites are collected and analyzed directly. Because daily dietary selenium intake of an individual can be highly variable, a series of diet collections provides a more precise estimate of habitual selenium intake than does a single collection (15). Additionally, the relation between tissue selenium and intake can be evaluated best by studying a population with a relatively wide range of selenium intake.

Adults from South Dakota and Wyoming were evaluated previously for evidence of selenium toxicity (16) because of their relatively high selenium intake. In that study a series of diet samples was collected and assayed for selenium content. In this report we examine the relation between dietary selenium and three potential indices of selenium status: serum, whole blood, and toenails. We also examined selenium intake and selenium status in relation to three factors—age, gender, and smoking status—reported to influence selenium status.

Methods

Subjects and protocol

Subjects were enrolled both from households selected at random and from households where unusually high selenium intake was suspected. The randomly selected subjects were chosen from western South Dakota and eastern Wyoming telephone directories. Subjects suspected of having high selenium intakes were from South Dakota ranches where selenosis in livestock had occurred.

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[2] Supported in part by a grant from the Selenium-Tellurium Development Association, Inc.

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Received September 27, 1989.

Accepted for publication December 27, 1989.

Am J Clin Nutr 1990;52:858–62. Printed in USA.

Potential subjects were contacted by mail and phone to explain the study and to invite participation. After the initial contact, individuals expressing interest in the study were visited in their homes, where the study was fully explained and informed consent was obtained. The protocol for the study included physical examinations, completion of health history and diet questionnaires, duplicate-plate diet collections for 2 d at 3-mo (quarterly) intervals, fasting 30-mL blood samples, and collections of toenail clippings and 24-h urine samples.

At the initial home visit subjects received a physical examination and were instructed in methods of recording diet and sample collection. The investigators and participants established a schedule for quarterly collections of diet samples and biological specimens. Subjects were asked to collect, for 2 nonconsecutive days each quarter, duplicate portions of all food and beverages consumed. Participants were instructed to schedule their food collections so that two of the eight collection days fell on Saturday or Sunday. Twenty-four–hour urine samples were collected to coincide with the second day of each quarterly diet collection. Quarterly blood donations were scheduled for the morning marking the completion of the 24-h urine collection. Participants were visited in their homes for blood collections. Containers for subsequent collections of diet and urine were distributed during those visits. Diet collections started in the summer of 1985 and ended in the spring of 1986.

Spouse pairs were asked to participate when more than one adult was present in a household. Only one person per household was assigned to collect diet composites. Of the 78 adults who participated in the study, 45 collected diet samples. This report is based on the analysis of data collected from 44 individuals (24 men and 20 women) who provided a minimum of six diet composites. The mean and median age of the diet collectors was 50 y (range 22–78 y). Four men and four women were smokers. All of the participants were white. Fifteen subjects lived on ranches where selenosis in livestock was suspected and these individuals consumed home produce. The subjects were judged to be in good health as determined from a medical evaluation. None of the subjects reported use of selenium supplements.

The study protocol was approved by human subjects committees of the US Department of Agriculture, the National Institutes of Health, and the Harvard School of Public Health.

Sample collection and analysis

Samples of diet, blood, and urine were collected and stored in plastic containers. Precautions were taken to avoid environmental contamination. The analytical blank for all samples averaged < 4 pmol Se.

Diet composites. Duplicate-plate food and beverage samples were collected as described elsewhere (17). Briefly, the subjects collected duplicate amounts of the edible portion of all foods and beverages consumed. Individual 24-h diet composites were weighed, homogenized, aliquoted, frozen, and shipped to Beltsville, MD, for processing and analysis.

Blood, urine, and toenails. Fasting venous blood samples were drawn into trace element-free Vacutainers (Becton Dickinson). Samples of whole blood and serum were obtained. Twenty-four–hour urine samples were collected directly into disposable plastic containers and processed as described elsewhere (17). Aliquots of 24-h urine collections, whole blood, and serum were frozen and shipped on dry ice to Beltsville, MD, for analysis. Subjects provided nail clippings from all toes.

TABLE 1
Selenium values of 44 adults residing in a seleniferous area of the United States*

Variable	
Se intake	
(μg/d)	174 ± 91 (68–444)
(μg/kg body wt)	2.33 ± 1.08 (0.98–5.10)
Se status indices	
Serum Se (μmol/L)	2.10 ± 0.38 (1.56–3.71)
Whole blood Se (μmol/L)	3.22 ± 0.79 (2.37–7.03)
Toenail Se (nmol/g)	15.2 ± 3.0 (10.4–27.9)
Urine Se	
(μmol/d)	1.56 ± 1.02 (0.3–5.0)
(nmol/kg body wt)	20.8 ± 12.4 (4.6–54.5)

* $\bar{x}$ ± SD; range in parentheses.

Nail samples were stored in envelopes and sent to J Steven Morris in Columbia, MO, for analysis.

Selenium determinations. Serum, whole blood, urine, and diet were analyzed for total selenium content by use of an isotope-dilution technique combined with gas chromatographic/mass spectrometric analysis. Methods of sample preparation, digestion, and analysis were described previously (18). The detection limit of the method (defined as three times the SD of the analytical blank) was 6 pmol Se. Quality control was maintained by regular determinations of in-house reference pools of serum, whole blood, and urine. The CV for duplicate analysis of the in-house reference pools was always < 2%. Toenail samples were cleaned and then analyzed for selenium content by neutron activation (19). Neutron-activation analysis of National Bureau of Standards Reference Material #1577 (bovine liver) was 14.1 ± 0.6 nmol/g ($\bar{x}$ ± SD) as compared with a certified value of 13.9 ± 1.3 nmol/g. The CV associated with 38 repeated measurements was 4.5%.

Statistical analysis

All subjects provided at least six diet composites, and most (77%) provided the complete set of eight. All subjects provided blood and toenail specimens for three quarters, and most of the subjects (89%) provided the complete set of four. Selenium concentration of diet, blood, and toenails did not vary seasonally (20); an average value for each indicator of selenium status was calculated for each subject.

Pearson correlation coefficients were computed for selenium intake vs selenium concentration of serum, whole blood, and toenails. Differences in selenium intake and selenium status by gender, current smoking status (no, yes), and age group (< 50 y, ≥ 50 y) were evaluated with the *t* test. Standard, normal-theory, linear multivariate-regression analysis was used to evaluate the relation between selenium status and selenium intake, gender, age, and smoking status. Statistical analyses were performed with *SAS* computer programs (21). Unless otherwise stated, all data were analyzed for statistical significance at the 5% significance level ($P < 0.05$).

Results

The average selenium intake of the 44 subjects and selenium concentrations in serum, whole blood, and toenails are given in **Table 1.** Urine selenium was ~70% of selenium intake, and

TABLE 2
Correlation between selenium intake and selenium in serum, whole blood, toenails, and urine*

	Serum	Blood	Toenails	Urine
	μmol/L	*μmol/L*	*nmol/g*	*μmol/d*
Se intake				
(μg/d)	0.51	0.55	0.53	0.87
(μg/kg body wt)	0.63	0.62	0.59	0.82

* Pearson correlation coefficients, all associations statistically significant ($P < 0.001$).

the two measures were highly correlated. For example, when mean intake and excretion of selenium were expressed per kilogram body weight, the Pearson correlation coefficient (r) was 0.84 ($P < 0.001$). Dietary selenium (μg/d, μg/kg body wt) was significantly correlated with selenium in serum, whole blood, and toenails (**Table 2**). The correlation of selenium intake and tissue selenium was greater when intake was expressed per kilogram body weight.

The average daily selenium intake of the men was 66% greater than that of the women (**Table 3**). When intake was expressed per kilogram body weight, the difference was less pronounced and marginally significant ($P = 0.05$). Despite higher dietary intakes, men had mean values of serum, whole blood, and toenail selenium that were similar to those of the women.

On average, the amount of selenium consumed by smokers was 35% less than that of nonsmokers. The difference increased slightly when intake was expressed as μg/kg body wt (**Table 4**). Smokers had mean values of serum, whole blood, and toenail selenium that were 12–23% lower than those of nonsmokers. Differences between smokers and nonsmokers were statistically significant for whole blood and toenails, but not serum.

Average selenium intake of individuals ≥ 50 y was slightly higher than that of younger subjects (**Table 5**) but group differences were not remarkable. Concentrations of selenium in serum, whole blood, and toenails were similar for both age categories.

Dietary selenium (μg/kg body wt) was the most important determinant of selenium in serum, whole blood, and toenails (**Table 6**). The regression coefficient associated with gender indicated that, for a given level of dietary selenium, women would have higher concentrations of selenium in serum, whole blood, and toenails than would men. The regression coefficient associated with age was negative but the inverse association between age and indices of selenium status was not statistically significant. Likewise, the regression coefficient associated with smoking was negative but statistically significant only when toenails were used as the index of selenium status.

TABLE 3
Selenium values of men and women residing in a seleniferous area of the United States*

Variable	Men ($n = 24$)	Women ($n = 20$)	P
Se intake			
(μg/d)	213 ± 97	128 ± 58	<0.001
(μg/kg body wt)	2.6 ± 1.2	2.0 ± 0.8	0.05
Se status indices			
Serum Se (μmol/L)	2.04 ± 0.27	2.17 ± 0.49	0.27
Whole blood Se (μmol/L)	3.14 ± 0.51	3.32 ± 1.03	0.49
Toenail Se (nmol/g)	14.8 ± 2.5	15.7 ± 3.7	0.34
Urine Se			
(μmol/d)	1.77 ± 1.05	1.28 ± 0.95	0.11
(nmol/kg body wt)	21.5 ± 12.7	20.3 ± 12.7	0.60

* $\bar{x} \pm$ SD.

TABLE 4
Selenium values of smokers and nonsmokers residing in a seleniferous area of the United States*

Variable	Smokers ($n = 8$)	Nonsmokers ($n = 36$)	P
Se intake			
(μg/d)	121 ± 46	186 ± 95	<0.01
(μg/kg body wt)	1.5 ± 0.4	2.5 ± 1.1	<0.001
Se status indices			
Serum Se (μmol/L)	1.87 ± 0.22	2.14 ± 0.39	0.07
Whole blood Se (μmol/L)	2.75 ± 0.32	3.33 ± 0.82	<0.01
Toenail Se (nmol/g)	12.2 ± 1.0	15.8 ± 2.9	<0.001
Urine Se			
(μmol/d)	0.91 ± 0.35	1.70 ± 1.08	<0.001
(nmol/kg body wt)	11.4 ± 3.8	22.8 ± 12.7	<0.001

* $\bar{x} \pm$ SD.

Discussion

Pennington et al (22) analyzed data from national food surveys and reported that the mean dietary selenium intake of adults in the United States was 108 μg/d. In this report we document a higher selenium intake (174 ± 91 μg/d, $\bar{x} \pm$ SD) among a group of South Dakota and Wyoming residents. The relatively high selenium intake of our population was undoubtedly related to consumption of local food items (23). The high selenium intake of our subjects was reflected by an unusually high average selenium concentration in serum (2.10 ± 0.38 μmol/L), whole blood (3.22 ± 0.79 μmol/L), and toenails (15.2 ± 3.0 nmol/g). In a multicenter European study (24), mean serum selenium ranged from a low of 0.8 μmol/L in Greece to a high of 1.4 μmol/L in London. In the United States whole blood

TABLE 5
Selenium values of young (< 50 y) and older (≥ 50 y) adults residing in a seleniferous area of the United States*

Variable	<50 y ($n = 22$)	≥50 y ($n = 22$)	P
Se intake			
(μg/d)	162 ± 79	187 ± 102	0.37
(μg/kg body wt)	2.24 ± 1.03	2.42 ± 1.14	0.59
Se status indices			
Serum Se (μmol/L)	2.10 ± 0.34	2.09 ± 0.43	0.89
Whole blood Se (μmol/L)	3.22 ± 0.58	3.22 ± 0.95	1.00
Toenail Se (nmol/g)	15.3 ± 2.3	15.1 ± 3.8	0.81
Urine Se			
(μmol/d)	1.51 ± 1.03	1.61 ± 1.06	0.75
(nmol/kg body wt)	20.6 ± 12.9	20.9 ± 12.2	0.96

* $\bar{x} \pm$ SD.

TABLE 6
Predictors of three indices of selenium status in adults residing in a seleniferous area (multivariate regression analysis)

Index and predictor variables*	β†	SEE	P	Total R^2‡
Serum Se (μmol/L)				
Se intake (μg/kg)	0.256	0.042	<0.0001	
Gender (female)	0.278	0.085	0.002	
Smoker (yes)	−0.038	0.110	0.74	
Age (y)	−0.005	0.003	0.14	
				0.57
Whole blood Se (μmol/L)				
Se intake (μg/kg)	0.498	0.092	<0.0001	
Gender (female)	0.461	0.188	0.02	
Smoker (yes)	−0.117	0.243	0.63	
Age (y)	−0.007	0.007	0.32	
				0.50
Toenail Se (nmol/g)				
Se intake (μg/kg)	1.68	0.35	<0.0001	
Gender (female)	1.98	0.71	0.01	
Smoker (yes)	−2.21	0.92	0.02	
Age (y)	−0.008	0.026	0.74	
				0.54

* Coding for regression variables: Se intake (continuous); gender (0, male; 1, female); smoker (0, no; 1, yes); age (continuous).

† The β coefficient indicates the specific influence on the dependent variable from a one unit change in the independent variable with the other regression variables held constant. For example, the serum Se concentration of a woman would be predicted to be 0.278 μmol/L higher than that of a man (with age, smoking, and Se intake constant).

‡ Total R^2 is the proportion of the variation explained by a linear model that includes all four predictor variables. R^2 for Se intake and serum Se, 0.40; for Se intake and whole blood Se, 0.38 and for Se intake and toenail Se, 0.35.

selenium ranged from a low of 2.0 μmol/L in Ohio (12) to a high of 3.4 μmol/L in a seleniferous part of South Dakota (25). Toenails have not been widely used to assess selenium status but samples from populations with low and high selenium exposure have been measured. Morris et al (19) reported mean values ranging from 3.3 nmol/g for New Zealand to 15.2 nmol/g for South Dakota residents.

Serum and whole blood selenium were associated with selenium intake in geographic correlation studies (11) but attempts to demonstrate similar associations within specific geographic sites were less successful. Snook et al (12), for example, determined the selenium content of 600 local food items and administered 24-h dietary recalls to Ohio residents. Plasma selenium was only weakly ($r = 0.12$) associated with calculated dietary selenium and whole blood selenium was not associated with intake. Using similar methods, Lane et al (13) reported that calculated dietary selenium was not associated with plasma selenium but was associated with erythrocyte selenium ($r = 0.38$). In our study of South Dakota adults, all three indices of selenium status were strongly associated with selenium intake. Our ability to demonstrate more clearly the association between selenium intake and tissue selenium content of free-living individuals can be attributed to direct analysis of several diet composites collected from a population having a relatively wide range of selenium intake.

In the multivariate analysis we could explain 50–57% of the variance in the indices of selenium status. Dietary intake explained the greatest portion of that variance. Seasonal sampling for 2 d was adequate to demonstrate an association between selenium intake and the selenium indices, but it is likely that additional diet collections and increased range of intake would have improved further the correlation between selenium exposure and selenium status. Therefore, the R^2 values reported here are probably underestimated. Animal and human studies both demonstrated that tissue selenium concentration is influenced by the chemical form of selenium in the diet (14). We were not able to consider the relative importance of chemical form vs amount of dietary selenium because information on the chemical form of selenium in foods is scarce; only wheat (26) and cabbage (27) have been characterized. Other dietary information was available from food frequency questionnaires but we were unable to identify any other single dietary variable (eg, intakes of protein, fat, fiber, energy, and methionine) that materially reduced the unexplained variance. Although others (8) reported lower selenium status among drinkers, alcohol intake of our subjects was relatively low and its distribution too limited to evaluate the relation between alcohol intake and selenium status.

Men consumed more food than did women, which accounted for their higher selenium intake. Although men had higher selenium intakes, their tissue selenium concentrations did not reflect higher exposure. To our knowledge there is no evidence that men absorb less selenium than do women, and men did not excrete proportionally more selenium in urine. Muscle probably forms the largest body pool of selenium (28). Men almost certainly require more selenium to maintain the same concentration of blood (or toenail) selenium as women with less muscle mass. In the multivariate analysis we expressed selenium intake in relation to body weight but we did not collect information that would allow us to adjust for differences in body composition. If we had been able to control for differences in lean body mass, it is likely that gender would not have been significant in the multivariate analysis.

Smokers consumed less selenium daily than did nonsmokers. Smokers consumed less food than did nonsmokers but this difference was not great enough to account for a 35% reduction of selenium intake. We calculated the amount of selenium per gram (dry weight) of diet consumed, which indicated that smokers consumed diets that were ~20% less concentrated in selenium. Dietary habits of smokers have not been adequately studied but there is other evidence that smokers have reduced food intakes and also select diets of low nutrient density (29). In the multivariate analysis smoking was no longer an important predictor of serum or whole blood selenium, suggesting that the lower selenium concentrations of smokers were explained by low dietary selenium rather than by smoking per se. Smoking, however, remained an independent determinant of selenium status when toenails were used as the index. Ellis et al (30) observed reduced serum and whole blood selenium concentrations of smokers. Lloyd et al (8) also reported reduced selenium status of male, but not female, smokers. Robinson et al (31) found no association between smoking and selenium status of New Zealand adults.

Food intake decreases as adults age (32). In our population of South Dakota and Wyoming residents, selenium intake of younger and older adults (< 50 vs ≥ 50 y) was similar. The regression coefficient associated with age was negative after selenium intake, smoking, and gender were controlled for, suggesting an inverse association. Age was not, however, significantly

predictive of the selenium indices. Dickson and Tomlinson (33) reported that plasma selenium decreased with age. Lloyd et al (8) observed that individuals aged > 55 y had reduced whole blood selenium concentration. They suggested that lower blood selenium of older individuals was due to less efficient absorption or increased excretion rather than to reduced intake. We measured urinary excretion of selenium and our older adults did not excrete more selenium. Other investigators have not found age-associated changes in selenium status of adults (6).

In summary, tissue selenium concentration was determined primarily by selenium intake. Despite higher selenium intake in men, their tissue selenium values were similar to those in women, an observation likely explained by differences in body composition. Smoking as a determinant of selenium status should be further explored but the lower tissue selenium concentrations of smokers in this study were related, at least in part, to lower selenium intake. Although we explained a substantial portion of the variance in tissue selenium concentration, almost half of the variation was left unexplained. It is likely that the unexplained variance could be reduced further by increasing both the range of selenium intake and the number of days of diet collections. The chemical form of dietary selenium undoubtedly accounts for some of the variance but the effect cannot be evaluated until the chemical forms of selenium in foods are characterized and quantified. Finally, all three indices of selenium status---serum, whole blood, and toenails---served equally well as indicators of selenium intake.

We thank Natalie Hardison, Kristine Patterson, and JS Morris for performing the selenium determinations. Elaine Wolfe contributed to data management and analysis, and Kathleen Corr assisted with study management and sample collection. We especially thank the participants from South Dakota and Wyoming for their cooperation.

References

1. Salonen JT, Alfthan G, Huttunen JK, Pikkarainen J, Puska PP. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. Lancet 1982;2:175–9.
2. Salonen JT, Alfthan G, Huttunen JK, Puska P. Association between serum selenium and the risk of cancer. Am J Epidemiol 1984;120:342–9.
3. Willett WC, Polk BF, Morris JS, et al. Prediagnostic serum selenium and risk of cancer. Lancet 1983;2:130–4.
4. Clark LC, Combs GF Jr. Selenium compounds and the prevention of cancer: research needs and public health implications. J Nutr 1986;116:170–3.
5. Burk RF. Selenium and cancer: meaning of serum selenium levels. J Nutr 1986;116:1584–6.
6. McAdam PA, Smith DK, Feldman EB, Hames C. Effect of age, sex, and race on selenium status of healthy residents of Augusta, Georgia. Biol Trace Elem Res 1984;6:3–9.
7. Miller L, Mills BJ, Blotcky AJ, Lindeman RD. Red blood cell and serum selenium concentrations as influenced by age and selected diseases. J Am Coll Nutr 1983;4:331–41.
8. Lloyd B, Lloyd RS, Clayton B. Effect of smoking, alcohol, and other factors on the selenium status of a healthy population. J Epidemiol Community Health 1983;37:213–7.
9. Thomson CD, Robinson MF, Campbell DR, Rea HM. Effect of prolonged supplementation with daily supplements of selenomethionine and sodium selenite or glutathione peroxidase activity in blood of New Zealand residents. Am J Clin Nutr 1982;36:24–31.
10. Levander OA, Alfthan G, Arvilomni H, et al. Bioavailability of selenium to Finnish men as assessed by platelet glutathione peroxidase activity and other blood parameters. Am J Clin Nutr 1983;37:887–97.
11. Levander OA. Clinical consequences of low selenium intake and its relationship to vitamin E. Ann NY Acad Sci 1982;393:70–80.
12. Snook JT, Palmquist DL, Moxon AL, Cantor AH, Vivian VM. Selenium status of a rural (predominantly Amish) community living in a low-selenium area. Am J Clin Nutr 1983;38:620–30.
13. Lane HW, Warren DC, Taylor BJ, Stool E. Blood selenium and glutathione peroxidase levels and dietary selenium of free-living and institutionalized elderly subjects. Proc Soc Exp Biol Med 1983;173:87–95.
14. Levander OA. The need for measures of selenium status. J Am Coll Toxicol 1986;5:37–44.
15. Mutanen M. Dietary intake and sources of selenium in young Finnish women. Hum Nutr Appl Nutr 1984;38A:265–9.
16. Longnecker MP, Taylor PR, Levander OA, et al. Tissue selenium (Se) levels and indices of Se exposure in a seleniferous area. Fed Proc 1987;46:587(abstr).
17. Levander OA, Morris VC. Dietary selenium levels needed to maintain balance in North American adults consuming self-selected diets. Am J Clin Nutr 1984;39:809–15.
18. Reamer DC, Veillon C. A double isotope dilution method for using stable selenium isotopes in metabolic tracer studies: analysis by gas chromatography/mass spectrometry (GC/MS). J Nutr 1983;113:786–92.
19. Morris JS, Stampfer MJ, Willett W. Dietary selenium in humans: toenails as an indicator. Biol Trace Elem Res 1983;5:529–37.
20. Taylor PR, Longnecker MP, Levander OA, et al. Seasonal variation in selenium (Se) status among free-living persons in South Dakota. Fed Proc 1987;46:882(abstr).
21. Statistical Analysis System Institute. SAS user's guide. Cary, NC: SAS Institute, Inc, 1982.
22. Pennington JAT, Wilson DB, Newell RF, Harland BF, Johnson RD, Vanderveen JE. Selected minerals in food surveys, 1974 to 1981/82. J Am Diet Assoc 1984;84:771–80.
23. Holden JM, Hardison NW, Salbe AD, Howe SM, Taylor PR, Longnecker MP. Selenium (Se) composition of self-selected diets and retail and homegrown foods in a seleniferous area of South Dakota. Fed Proc 1988;2:A1622(abstr).
24. Thorling EB, Overvad K, Geboers J. Selenium status in Europe-human data a multicenter study. Ann Clin Res 1986;18:3–7.
25. Howe SM. Selenium in blood of South Dakotans. Arch Environ Health 1979;34:444–8.
26. Olson OE, Novacek EJ, Whitehead EI, Palmer IS. Investigations on selenium in wheat. Phytochemistry 1970;9:1181–8.
27. Hamilton JW. Chemical examination of seleniferous cabbage *Brassica oleracea capitata*. J Agric Food Chem 1975;23:1150–2.
28. Behne D, Wolters W. Distribution of selenium and glutathione peroxidase in the rat. J Nutr 1983;113:456–61.
29. Fehily AM, Phillips KM, Yarnell JWG. Diet, smoking, social class, and body mass index in the Caerphilly Heart Disease Study. Am J Clin Nutr 1984;40:827–33.
30. Ellis N, Lloyd B, Lloyd RS, Clayton BE. Selenium and vitamin E in relation to risk factors for coronary heart disease. J Clin Pathol 1984;37:200–6.
31. Robinson MF, Campbell DR, Sutherland WHF, Herbison GP, Paulin JM, Simpson FO. Selenium and risk factors for cardiovascular disease in New Zealand. NZ Med J 1983;12:755–7.
32. Munro HN. Aging and nutrition: a multifaceted problem. In: Hutchinson ML, Munro HN, eds. Nutrition and aging. Orlando, FL: Academic Press, 1986:1–10.
33. Dickson RC, Tomlinson RH. Selenium in blood and human tissues. Clin Chim Acta 1967;16:311–21.